Tree Physiology 21, 541–547

Soil temperature and plant growth stage influence nitrogen uptake and amino acid concentration of apple during early spring growth

SHUFU DONG,[1] CAROLYN F. SCAGEL,[2] LAILIANG CHENG,[3] LESLIE H. FUCHIGAMI[1,5] and PAUL T. RYGIEWICZ[4]

[1] *Department of Horticulture, Oregon State University, Corvallis, OR 97331, USA*

[2] *USDA-ARS, Horticultural Crops Research Laboratory, 3420 NW Orchard Street, Corvallis, OR 97330, USA*

[3] *Department of Fruit and Vegetable Science, 134A Plant Science, Cornell University, Ithaca, NY 14853, USA*

[4] *U.S. EPA, National Health and Environmental Effects Research Laboratory, Western Ecology Division, 200 SW 35th Street, Corvallis, OR 97333, USA*

[5] *Author to whom correspondence should be addressed*

Received August 22, 2000

Summary In spring, nitrogen (N) uptake by apple roots begins about 3 weeks after bud break. We used 1-year-old 'Fuji' *Malus domestica* Borkh on M26 bare-root apple trees to determine whether the onset of N uptake in spring is dependent solely on the growth stage of the plant or is a function of soil temperature. Five times during early season growth, N uptake and total amino acid concentration were measured in trees growing at aboveground day/night temperatures of 23/15 °C and belowground temperatures of 8, 12, 16 or 20 °C. We used $^{15}NH_4{}^{15}NO_3$ to measure total N uptake and rate of uptake and found that both were significantly influenced by both soil temperature and plant growth stage. Rate of uptake of ^{15}N increased with increasing soil temperature and changed with plant growth stage. Before bud break, ^{15}N was not detected in trees growing in the 8 °C soil treatment, whereas ^{15}N uptake increased with increasing soil temperatures between 12 and 20 °C. Ten days after bud break, ^{15}N was still not detected in trees growing in the 8 °C soil treatment, although total ^{15}N uptake and uptake rate continued to increase with increasing soil temperatures between 12 and 20 °C. Twenty-one days after bud break, trees in all temperature treatments were able to acquire ^{15}N from the soil, although the amount of uptake increased with increasing soil temperature. Distribution of ^{15}N in trees changed as plants grew. Most of the ^{15}N absorbed by trees before bud break (~5% of ^{15}N supplied per tree) remained in the roots. Forty-six days after bud break, approximately one-third of the ^{15}N absorbed by the trees in the 12–20 °C soil temperature treatments remained in the roots, whereas the shank, stem and new growth contained about two-thirds of the ^{15}N taken up by the roots. Total amino acid concentration and distribution of amino acids in trees changed with plant growth stage, but only the amino acid concentration in new growth and roots was affected by soil temperature. We conclude that a combination of low soil temperature and plant developmental stage influences the ability of apple trees to take up and use N from the soil in the spring. Thus, early fertilizer application in the spring when soil temperatures are low or when the aboveground portion of the tree is not actively growing may be ineffective in promoting N uptake.

Keywords: developmental stage, ^{15}N, Malus domestica, roots.

Introduction

Application of fertilizers to soil in the early spring when soil temperatures are low is often undertaken during establishment of nursery stock plants and in commercial orchards (Westwood 1988, Toselli et al. 1999). Soil temperature influences water and nutrient uptake, metabolic processes and root and shoot growth (Hogue and Neilsen 1986, Tagliavini et al. 1991, Engels and Marschner 1992, Marshner 1995, McMichael and Burke 1998, Toselli et al. 1999). In many plant species, nutrient uptake by roots decreases at low root zone temperatures (Hogue and Neilsen 1986, Power and Zachariassen 1993, McCallister et al. 1997, Toselli et al. 1999, Vasilieva et al. 1999, Weih and Karlsson 1999). Nutrient uptake is also closely related to the physiological status, nutrient status, and nutrient requirements of the plant, all of which change with the plant developmental stage (Memon et al. 1988, Awonaike et al. 1991, Knowles et al. 1991, Marti and Mills 1991*a*, 1991*b*, Alcoz et al. 1993, Karrou and Maranvill 1994, Marschner 1995). Cheng and Fuchigami (1997) reported that, in apple trees growing under natural conditions, nitrogen (N) uptake from the soil was detectable 21 days after bud break in the spring; however, they did not determine whether the onset of N uptake is influenced more by soil temperature or plant developmental stage. The possible interaction between soil temperature and plant growth stage on nutrient uptake suggests that timing of fertilizer application with respect to plant growth stage is an important consideration when attempting to optimize nutrient uptake and use.

Initial growth of woody plants is supported by remobil-

ization of storage reserves accumulated in the previous year. In apple trees, reserve N is remobilized in the spring from storage sites to growing tissues, mainly in amino acid forms (Tromp and Ovaa 1971, 1973, O'Kennedy et al. 1975, Kang et al. 1981). It is not known whether this remobilization of amino acids affects the onset of N uptake in the early spring.

Some nurseries and commercial orchards apply urea to leaves of plants in the fall to promote growth during the following spring (Shim et al. 1972, 1973, Rosecrance et al. 1998, Tagliavini et al. 1998, Khemira et al. 2000). Application of N in the fall increases N reserves and changes amino acid concentrations and distribution of both immediately after application and during the early stages of growth the following spring. It is not known if these shifts in amino acid concentration and N use within the trees are a result of changing plant developmental status or the influence of external factors, such as temperature.

In this study, we tracked early season changes in ^{15}N uptake and amino acid distribution at different root zone temperatures to test whether (1) temperature effects on N uptake are influenced by plant growth stage, and (2) changes in amino acid distribution are related to N uptake.

Materials and methods

Plant culture

In early spring (March 20, 1999), 1-year-old *Malus domestica* Borkh var. 'Fuji' on M26 bareroot apple trees with intact root systems were planted in 2-l pots containing a 1:1 (v/v) mix of perlite:vermiculite and placed in a greenhouse (day/night temperature of 25/20 °C and natural photoperiod) in Corvallis, OR. During the experiment, 120 ml of Hoagland's solution (Taiz and Zeiger 1998) was applied to each pot every 5 days, and 150 ml water was applied to each pot between nutrient solution applications during the later phase of the study.

Experimental setup

Trees were randomly divided into five groups, with 20 plants in each group. At 0, 14, 25, 38 and 50 days after planting, one group of trees was moved into growth chambers (day/night temperature of 23/15 °C and 12-h photoperiod of 200 µmol $m^{-2}s^{-1}$), and the pots were placed in controlled-temperature boxes with the stem portion exposed to the temperature of the growth chamber and the shank portion of the stem insulated with a 2.5-cm foam barrier. Four plants were placed in each box and temperatures inside the boxes were maintained at 8, 12, 16 or 20 °C.

After the temperature in the root boxes had stabilized (approximately 2 days), controls received 120 ml of Hoagland's solution without N, whereas the remainder of the plants received the same amount of Hoagland's solution labeled with 1 g of $^{15}NH_4{}^{15}NO_3$ (0.03% ^{15}N atom depleted, ISOTEC, Inc., Miamisburg, OH).

Harvest and measurements

Five days after ^{15}N application, plants in the growth chambers were harvested and divided into roots, shanks (rootstock tissue between roots and grafting union), stems (previous years scion above the shank and before new growth) and new shoots (including new stem and leaves). All samples were freeze-dried and ground with a Wiley Mill. Total amino acids were determined by the ninhydrin assay (Yemm and Cocking 1955) and absorbance at 580 nm was measured spectrophotometrically (UV-160, Shimadzu Corporation, Kyoto, Japan). Results were calculated in mg l^{-1} from a standard curve then multiplied by the total extraction volume to obtain amino acid concentrations in tissues expressed in mg kg^{-1}. Total N was determined by Kjedahl analysis (Lang 1958) by the Central Analysis Lab of Oregon State University. The amount of ^{15}N in samples was determined from the gas evolved from combustion of powdered tissue in an elemental analyzer coupled with a mass spectrometer (CARLO ERBA NC 2500, Carlo-Erba/Fisons Instruments, Valencia, CA). The percentage of N derived from fertilizer in plant tissues (NDFF%) was calculated as:

$$\text{NDFF\%} = \frac{(\text{atom\% } ^{15}\text{N})_{\text{natural abundance}} - (\text{atom\% } ^{15}\text{N})_{\text{tissue}}}{(\text{atom\% } ^{15}\text{N})_{\text{natural abundance}} - (\text{atom\% } ^{15}\text{N})_{\text{fertilizer}}} 100\%$$

Content of ^{15}N was calculated from NDFF% and total tissue N, and amount of ^{15}N in each tissue type was calculated by multiplying ^{15}N content by the dry weight of the tissue. Uptake of ^{15}N per plant was calculated by pooling the ^{15}N in different tissues, and ^{15}N uptake rate was calculated by dividing the total amount of ^{15}N by the time period over which uptake was measured.

Statistical analyses

The experiment was a two-factor (five growth stages and four soil temperatures), randomized block design with four replicates in each treatment. Data were subjected to a 2-factor analysis of variance (ANOVA), and linear correlations between NDFF% and total amino acid concentration were calculated based on the Pearson Correlation Coefficient (r). All statistical analyses were performed with NCSS'97 Statistical System Software (NCSS Statistical Analysis Software, Kaysville, UT).

Results

Plant growth

One week after trees were placed in the greenhouse, buds on the upper portion of the shoots began to swell, and after approximately 11 days in the greenhouse, buds broke and new shoots and leaves continued to elongate for the remainder of the experiment (Figure 1).

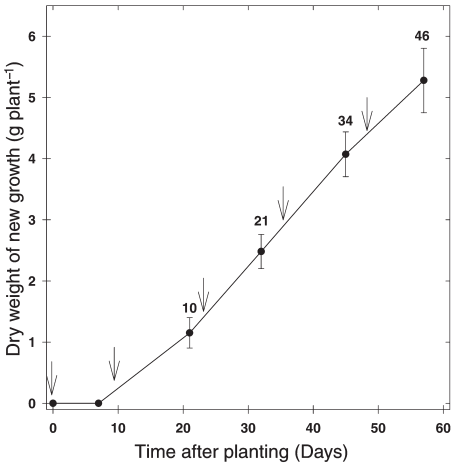

Figure 1. Whole-plant dry weight increment in 'Fuji'/M26 apple trees during the first 57 days after planting. Arrows indicate when a group of trees was placed in controlled temperature root boxes in growth chambers. Numbers above data points correspond to the mean number of days after bud break. Bars on data points represent standard errors of the mean of 16 replicates.

Total ^{15}N uptake and uptake rate

Total uptake of ^{15}N was significantly influenced by both soil temperature and plant growth stage ($F_{9,32}$ = 3.46, MSE = 0.001009, $P < 0.001$). At low soil temperature (8 °C), uptake of ^{15}N was first detected 32 days after planting (21 days after bud break) (Figure 2). In contrast, in the highest soil temperature treatment (20 °C), ^{15}N uptake was detected about 7 days after planting (4 days before bud break). The rate of ^{15}N uptake varied with both soil temperature and plant growth stage ($F_{9,32}$ = 4.15, MSE = 0.00081, $P < 0.001$). Uptake rate of ^{15}N increased with increasing soil temperature and increased as trees developed (Figure 3). The difference between ^{15}N uptake rates at low and high soil temperature treatments increased as trees developed.

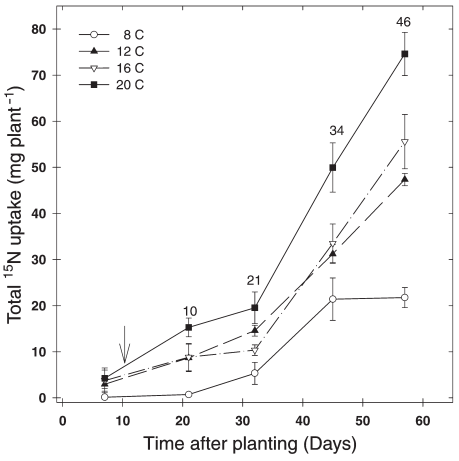

Figure 2. Total ^{15}N uptake of 'Fuji'/M26 apple trees at four temperatures during the 57 days after planting. Numbers above data points correspond to the mean number of days after bud break. Arrow represents the mean number of days after planting for bud break. Bars on data points represent standard errors of the mean of four replicates.

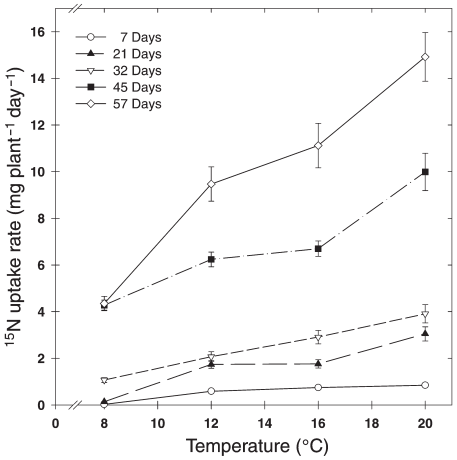

Figure 3. Influence of temperature on ^{15}N uptake rate of 'Fuji'/M26 apple trees 7, 21, 32, 45 and 57 days after planting. Bars on data points represent standard errors of the mean of four replicates.

Distribution of ^{15}N

The percentage of ^{15}N in tissues from the shank, stem and new growth was significantly influenced by both soil temperature and plant developmental stage (shank $F_{15,48}$ = 3.32, MSE = 0.00097, $P < 0.01$; stem $F_{15,48}$ = 2.34, MSE = 0.000283 $P < 0.05$; new growth $F_{15,48}$ = 2.67, MSE = 0.0062, $P < 0.05$). In

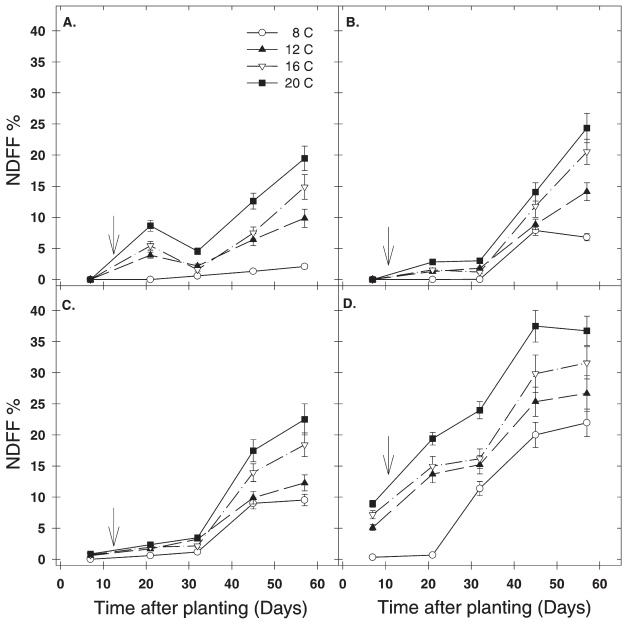

Figure 4. The NDFF% (N derived from fertilizer) in tissue from (A) new growth, (B) stem, (C) shank and (D) roots of 'Fuji'/M26 apple trees at four soil temperatures, during the 57 days after planting. Arrow represents the mean number of days after planting until bud break. Bars on data points represent standard errors of the mean of four replicates.

the 20 °C soil treatment, the percentage of ^{15}N detected in tissue from the previous season (shank and stem portions) was relatively low compared with the percentage of ^{15}N in roots (Figure 4). During the first 21 days after bud break, a higher percentage of ^{15}N was detected in tissue from new growth compared with previous-year stem and shank tissues. Between 21 and 46 days after bud burst, when new growth was well underway, the increase in percentage of ^{15}N in new growth and previous-season tissues was similar, but it was still lower than the percentage of ^{15}N in root tissue. The amount of ^{15}N in roots increased throughout the experiment ($F_{3,48}$ = 47.01, MSE = 0.0046, $P < 0.01$), and increased with increasing soil temperature ($F_{3,48}$ = 16.45, MSE = 0.046, $P < 0.01$).

Amino acid concentration and distribution

In general, soil temperature had little effect on the total amino acid concentration of tissues from the shank and stem (shank $F_{3,48}$ = 1.96, MSE = 0.133, $P > 0.05$, stem $F_{3,48}$ = 2.98, MSE = 0.197, $P > 0.05$), whereas amino acid concentration of roots and new growth increased with increasing soil temperature (root $F_{3,48}$ = 3.94, MSE = 0.125, $P < 0.05$, new growth $F_{3,48}$ = 12.94, MSE = 0.121, $P < 0.01$) (Figure 5). The distribution of amino acids in trees changed significantly with plant growth stage ($F_{15,192}$ = 25.09, MSE = 0.093, $P < 0.01$), but only the amino acid concentration in new growth was significantly affected by both soil temperature and growth stage ($F_{15,48}$ = 2.97, MSE = 0.121, $P < 0.01$). Before bud break, the root, shank and stem portions of the trees contained similar amounts of amino acids. After bud break, amino acid concentrations in roots and shanks increased slightly then remained constant during the next 46 days, whereas the amino acid concentration in stems increased during the first 21 days after bud break then declined. Thirteen days after bud break, the amino acid concentration in new growth increased with increasing soil temperature. Although total amino acid concentration in new growth decreased between 21 to 46 days after bud break, the treatment differences in amino acid concentrations were maintained.

Relationship between amino acid concentration and NDFF%

At all root zone temperatures, NDFF% and amino acid concentration were poorly correlated during the first 32 days after planting, but significantly correlated at 45 and 57 days after planting (Table 1). Correlations between NDFF% and amino acid concentration in the different portions of the trees (root, shank, stem and new growth) were also poorly correlated during the first 21 days after bud break, but significantly correlated at 34 and 46 days after bud break (Table 2). Amino acid concentration and NDFF% were significantly correlated in new growth and roots at high root zone temperatures and poorly correlated in the stem and shank (Table 3, Figure 6).

Discussion

Soil temperature can strongly influence root initiation, root growth and nutrient uptake, and subsequently impact shoot development and mineral nutrient accumulation of plants (Hogue and Neilsen 1986, Tagliavini et al. 1991, McMichael

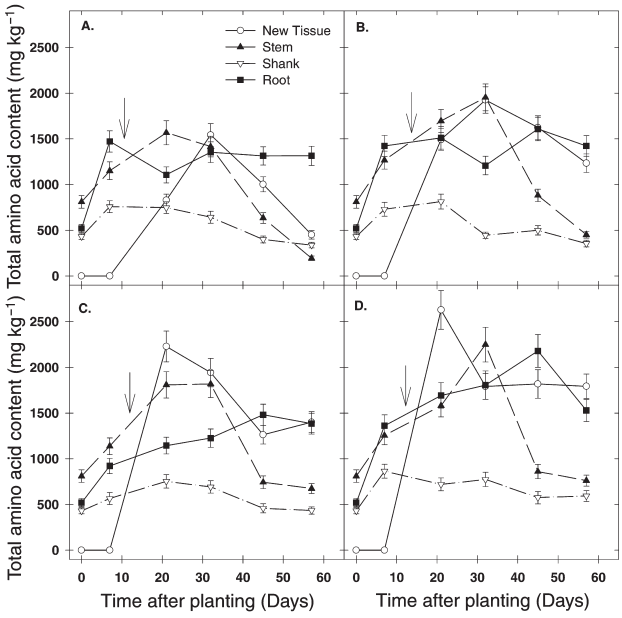

Figure 5. Total amino acid concentration in roots, shanks, stems and new growth of 'Fuji'/M26 apple trees at (A) 8 °C, (B) 12 °C, (C) 16 °C and (D) 20 °C, during 57 days after planting. Arrow represents the mean number of days after planting until bud break. Bars on data points represent standard errors of the mean of four replicates.

Table 1. Pearson correlation coefficients (r) and P-values between total amino acid concentration and NDFF% (N derived from fertilizer) of 'Fuji'/M26 apple trees at different root zone temperatures 7, 21, 32, 45 and 57 days after planting (4 days before, and 10, 21, 34 and 46 days after bud break, respectively).

Soil temperature (°C)	Day	r	P
8	7	0.489	0.107
	21	0.477	0.117
	32	0.234	0.463
	45	0.714	0.009
	57	0.951	0.000
12	7	0.476	0.118
	21	0.312	0.324
	32	0.244	0.445
	45	0.809	0.001
	57	0.950	0.000
16	7	0.147	0.648
	21	0.344	0.273
	32	0.093	0.744
	45	0.856	0.000
	57	0.962	0.000
20	7	0.511	0.090
	21	0.290	0.425
	32	0.136	0.674
	45	0.947	0.000
	57	0.882	0.000

Table 2. Pearson correlation coefficients (*r*) and *P*-values between total amino acid concentration and NDFF% (N derived from fertilizer) in different tree parts of 'Fuji'/M26 apple trees 7 21, 32, 45 and 57 days after planting (4 days before, and 10, 21, 34 and 46 days after bud break, respectively).

Tree part	Day	*r*	*P*
New growth	7	—	—
	21	0.833	0.001
	32	0.367	0.241
	45	0.818	0.001
	57	0.912	0.000
Stem	7	—	—
	21	0.301	0.342
	32	0.563	0.057
	45	0.202	0.528
	57	0.722	0.008
Shank	7	–0.161	0.619
	21	0.154	0.633
	32	0.412	0.183
	45	0.617	0.033
	57	0.750	0.005
Root	7	–0.188	0.558
	21	0.510	0.090
	32	0.473	0.121
	45	0.848	0.001
	57	0.751	0.005

Table 3. Pearson correlation coefficients (*r*) and *P*-values between total amino acid concentration and NDFF% (N derived from fertilizer) in different tree parts of 'Fuji'/M26 apple trees growing at different root zone temperatures.

Tree part	Soil temperature (°C)	*r*	*P*
New growth	8	0.470	0.049
	12	0.687	0.002
	16	0.541	0.020
	20	0.737	0.000
Stem	8	0.390	0.139
	12	–0.458	0.056
	16	–0.397	0.108
	20	–0.235	0.348
Shank	8	–0.345	0.220
	12	–0.131	0.604
	16	–0.338	0.170
	20	–0.128	0.612
Root	8	0.372	0.128
	12	0.670	0.002
	16	0.832	0.000
	20	0.685	0.002

and Burke 1998). We found that, in young Fuji/M26 apple trees, low soil temperature (8 °C) reduced uptake of ^{15}N by roots. At low temperatures, the viscosity of water increases causing a decrease in water flow to the root, and root permeability and metabolic activity decrease (Bowen 1991, Pavel and Fereres 1998). At low temperatures, the structure of membrane lipids in roots also changes (Simon 1974), and the activities of enzymes on the membrane responsible for nutrient uptake such as H^+-ATPase decrease (Ryyppo et al. 1994). In general, low soil temperature will reduce root function and therefore nutrient uptake will decrease concomitantly.

Toselli et al. (1999) reported that low root zone temperature (LRT) reduced the rate of N uptake by non-bearing, 1-year-old rooted cuttings of 'Mark' apple trees compared with high root zone temperature (HRT) on the day after ^{15}N fetilization, whereas uptake rates were similar for both LRT and HRT at 2, 4 and 8 days after fertilization. We found that low soil temperature decreased ^{15}N uptake by 1-year-old 'Fuji'/M26 apple trees for at least 5 days after ^{15}N application. The differences in sensitivity of ^{15}N uptake to temperature between our study and that of Tosselli et al. (1999) may be associated with growth stage, cultivar or culture conditions. Toselli et al. (1999) measured N uptake of plants that had been grown in a greenhouse for 60 days, whereas our measurements were made within 57 days after 1-year-old plants were put in a greenhouse.

Physiological status influences the nutrient demand of plants and subsequently affects nutrient uptake (Mariti and Mills 1991*a*, 1991*b*, Guindo et al. 1994, Hanson and Howell 1995, Bashir et al. 1997, Scoggins and Mills 1998, Wilson et al. 1998). Cheng and Fuchigami (1997) described differences

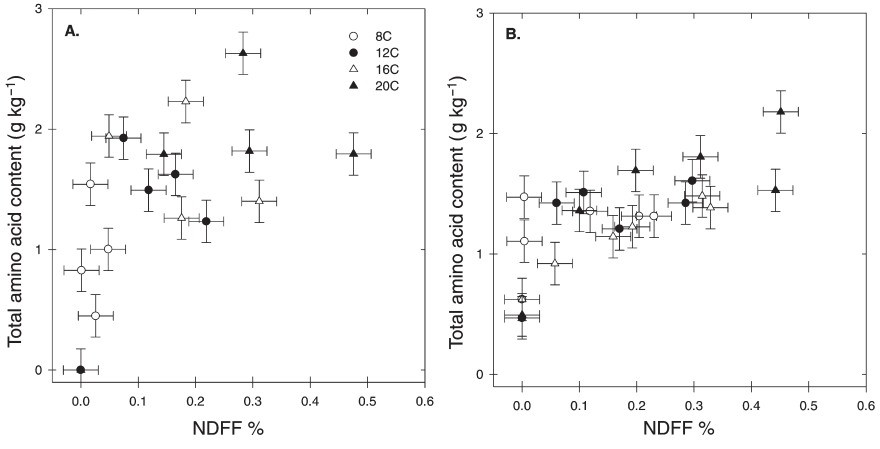

Figure 6. Relationship between total amino acid concentration and NDFF% (N derived from fertilizer) of 'Fuji'/M26 apple trees for (A) new growth, (B) roots at different root zone temperatures. Bars on data points represent standard errors of the mean.

in N uptake with development. We found significant differences in ^{15}N uptake as trees developed and no significant uptake of ^{15}N was detected at the lowest root temperature tested (8 °C) until 21 days after bud break.

The developmental stage of the plant not only influences nutrient uptake but also the partitioning of absorbed elements. Toselli et al. (2000) reported that when N was supplied to apple trees in summer, most N was partitioned to roots and 2- to 4-year-old wood, whereas when N was supplied in the spring it was partitioned to fruit and leaves and only a small amount was detected in the roots. We found that the translocation of absorbed ^{15}N changed with plant growth stage, and little ^{15}N was transferred from roots to other tissues in the early growth stage at low soil temperatures.

Early season growth of deciduous trees is supported by N remobilized from previous-year reserves (Titus and Kang 1982, Weinbaum et al. 1984, Millard and Neilsen 1989, Neilsen et al. 1997). Most N remobilization occurs before there is much root uptake of N (Millard and Neilsen 1989, Millard and Proe 1991). The relative contributions of N reserves and current uptake of N to annual tree growth depend on environmental conditions and the tree growth stage. During the early growth stages, reserve N contributed more to new growth than current uptake, but the contribution of current uptake increased as growth proceeded (Figure 4).

Amino acids are the primary products of N assimilation, and they are the main forms of N tranlocated in plants (Titus and Kang 1982). Amino acids in xylem sap of apple shoots vary substantially, depending on the stage of shoot development and the timing of N applications (Cooper et al. 1976). Because reserve N is converted to amino acids in early spring, making it available for the new growth (Kang et al. 1981, Titus and Kang 1982), changes in amino acid concentrations at this time are thought to result mainly from hydrolysis of N reserves and not from current N uptake. We found no significant correlation between amino acid concentration and current ^{15}N uptake in the early stages of plant development (Tables 1 and 2). However, as plant development proceeded, new growth became more dependent on current uptake of N (Figure 4), and the correlation between amino acid concentration and ^{15}N uptake (NDFF%) became more significant at the later stages of the experiment (Tables 1 and 2). Therefore, we concluded that, during the later developmental stages, the increase in amino acids was a result of NDFF%, whereas during the early stages changes in amino acid concentration were caused by remobilization of reserve N.

New growth (including both new shoots and roots) in the early spring is strongly dependent on temperature (Westwood 1988). New tissues act as strong sinks for N (Faust 1989). Once current uptake of ^{15}N is assimilated into amino acids, these amino acids are translocated into new tissues. This may explain why soil temperature influenced the relationship between amino acid concentration and NDFF% only for new tissues and roots (Table 3 and Figure 6).

In summary, there was a significant interaction between soil temperatures and growth stage on N uptake by young 'Fuji' apple trees on M26 rootstocks. Low soil temperature had less effect on N uptake of relatively inactive trees than of actively growing trees; however, after bud break low soil temperature had an increasingly pronounced effect on N uptake. We conclude that a combination of low soil temperature and plant developmental stage influences the ability of apple plants to take up and use N from the soil in the spring, and that early fertilizer application in the spring when soil temperatures are low may not be effective promoting N uptake. Amino acids in tissues are mainly derived from reserve N in the early stages of plant development, but amino acid synthesis becomes increasingly dependent on current uptake of N as plant development proceeds.

Acknowledgments

This work was funded in part by Washington Tree Fruit Research Commission, Northwest Nursery Improvement Institute, Oregon Association of Nurserymen and USDA-ARS. The authors thank Dr. Eric Hobbie for assistance with ^{15}N analysis and the U.S. EPA, National Health and Environmental Effects Research Laboratory, Western Ecology Division, for providing facilities for ^{15}N analysis.

References

Alcoz, M.M., F.M. Hons and V.A. Haby. 1993. Nitrogen fertilization timing effects on wheat production, nitrogen uptake efficiency, and residual soil nitrogen. Agron. J. 85:1198–1203.

Awonaike, K.O., K.S. Kumarasinghe and S.K.A. Danso. 1991. Nitrogen assimilation and distribution in field-grown cowpea at various growth stages. Soil Sci. Soc. Am. J. 55:81–85.

Bashir, R., R.J. Norman, R.K. Bacon and B.R. Wells. 1997. Accumulation and redistribution of fertilizer nitrogen-15 in soft red winter wheat. Soil Sci. Soc. Am. J. 61:1407–1412.

Bowen, G.D. 1991. Soil temperature, root growth, and plant function. *In* Plant Root, the Hidden Half. Eds. Y. Waisel, A. Eshel and U. Kafkafi. Marcel Dekker, New York, pp 309–330.

Cheng, L. and L.H. Fuchigami. 1997. Regrowth performance of apple nursery plants in relation to reserve and current uptake nitrogen. Annual Progress Report for Washington Tree Fruit Research Commission, Northwest Nursery Improvement Institute, Oregon Association of Nurserymen., pp 14–21.

Cooper, D.R., D.G. Hill-Cottingham and C.P. Lloyd-Jones. 1976. Distribution and identity of labeled products following autumn application of ^{15}N-labeled urea or potassium nitrate to apple trees. J. Food Agri. Sci. 27:266–272.

Engels, C. and H. Marschner. 1992*a*. Root to shoot translocation of macronutrients in relation to shoot demand in maize (*Zea mays* L.) grown at different root zone temperature. Z. Pflanzen. Boden. 155:121–128.

Faust, M. 1989. Physiology of temperate zone fruit trees. John Wiley and Sons, New York, pp 53–132.

Guindo, D., R.J. Norman and B.R. Wells. 1994. Accumulation of fertilizer nitrogen-15 by rice at different stages of development. Soil Sci. Soc. Am. J. 58:410–415.

Hanson, E.J. and G.S. Howell. 1995. Nitrogen accumulation and fertilizer use efficiency by grapevines in short-season growing areas. HortScience 30:504–507.

Hogue, E.J. and G.H. Neilson. 1986. Effect of root temperature and varying cation ratios on growth and leaf cation concentration of apple seedlings grown in nutrient solution. Can. J. Plant Sci. 66: 637–645.

Kang, S.M., K.C. Ko and J.S. Titus. 1981. Proteolysis in apple shoot bark during spring growth. Plant Physiol. 67:86.

Karrou, M. and J.W. Maranville. 1994. Response of wheat cultivars to different soil nitrogen and moisture regimes. II. Nitrogen uptake, partitioning and influx. J. Plant Nutr. 17:745–761.

Khemira, H., E. Sanchez, T.L. Righetti and A. Azarenko. 2000. Phytotoxity of urea and biuret sprays to apple foliage. J. Plant Nutr. 23:35–40.

Knowles, T.C., T.A. Doerge and M.J. Ottman. 1991. Improved nitrogen management in irrigated durum wheat using stem nitrate analysis. I. Nitrate uptake dynamics. Agron. J. 83:346–352.

Lang, C.A. 1958. Simple micro-determination of Kjeldahl nitrogen in biological materials. Anal. Chem. 30:1692–1694.

Marti, H.R. and H.A. Mills. 1991*a*. Calcium uptake and concentration in bell pepper plants as influenced by nitrogen form and stages of development. J. Plant Nutr. 14:1177–1185.

Marti, H.R. and H.A. Mills. 1991*b*. Nutrient uptake and yield to sweet pepper as affected by stage of development and N form. J. Plant Nutr. 14:1165–1175.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, London, pp 131–201.

McCallister, D.L., L.A. Jawson and M.D. Jawson. 1997. Soil temperature and fumigation effects on plant phosphorus uptake and related microbial properties. J. Plant Nutr. 20:485–497.

McMichael, B.L. and J.J. Burke. 1998. Soil temperature and root growth. HortScience 33:947–951.

Memon, Y.M., I.F. Fergus, J.D. Hughes and D.W. Page. 1988. Utilization of non-exchangeable soil potassium in relation to soil type, plant species, and stage of growth. Aust. J. Soil Res. 26:489–496.

Millard, P. and G.H. Neilsen. 1989. The Influence of nitrogen supply on the uptake and remobilization of stored N for the seasonal growth of apple trees. Ann. Bot. 63:301–309.

Millard, P. and M.F. Proe. 1991. Leaf demography and the seasonal internal cycling of nitrogen in sycamore *(Acer pseudoplatanus* L.) seedlings in relation to nitrogen supply. New Phytol. 117: 587–596.

Neilsen, D., P. Millard, G.H. Neilsen and E.J. Hogue. 1997. Sources of N for leaf growth in a high-density apple (*Malus domestica*) orchard irrigated with ammonium nitrate solution. Tree Physiol. 17:733–739.

O'Kennedy, B.T., M.J. Hennerty and J.S. Titus. 1975 Changes in the nitrogen reserves of apple shoots during the dormant season. J. Hort. Sci. 50:321–329.

Pavel, E.W. and E. Fereres. 1998. Low soil temperatures induce water deficits in olive (*Olea europaea*) trees. Physiol. Plant. 104: 525–532.

Power, J.F. and J.A. Zachariassen. 1993. Relative nitrogen utilization by legume cover crop species at three soil temperatures. Agron. J. 85:134–140.

Rosecrance, R.C., R.S. Johnson and S.A. Weinbaum. 1998. The effect of timing of post-harvest foliar urea sprays on nitrogen absorption and partitioning in peach and nectarine trees. J. Hort. Sci. Biotech. 73:856–861.

Ryyppo, A., E.M. Vapaavuori, R. Rikala and M.-L. Sutinen. 1994. Fatty acid composition of microsomal phospholipids and H^+-ATPase activity in roots of Scots pine seedlings grown at different root temperatures during flushing. J. Exp. Bot. 45: 1522–1539.

Scoggins, H.L. and H.A. Mills. 1998. Poinsettia growth, tissue nutrient concentration, and nutrient uptake as influenced by nitrogen form and stage of growth. J. Plant Nutr. 21:191–198.

Shim, K., J.S. Titus and W.E. Splittstoesser. 1972. The utilization of post-harvest urea sprays by senescing apple leaves. J. Am. Soc. Hort. Sci. 97:592–596.

Shim, K., J.S. Titus and W.E. Splittstoesser. 1973. The fate of Carbon and nitrogen from urea applied to foliage of senescing apple trees. J. Am. Soc. Hort. Sci. 98:360–366.

Simon, E.W. 1974. Phospholipids and plant membrane permeability. New Phytol. 73:377–420.

Tagliavini, M., E.J. Hogue and G.H. Neilson. 1991. Influence of phosphorus nutrition and root zone temperature on growth and mineral uptake of peach seedlings. J. Plant Nutr. 14:1267–1276.

Tagliavini, M., P. Millard and M. Quartieri. 1998. Storage of foliar-absorbed nitrogen and remobilization for spring growth in young nectarine (*Prunus persica* var. *nectarina*) trees. Tree Physiol. 18:203–207.

Taiz, L. and E. Zeiger. 1998. Plant physiology, 2nd Edn. Sinauer Associates Inc., Publishers, Sunderland, MA, pp 103–124.

Titus, J.S. and S. Kang. 1982. Nitrogen metabolism, translocation and recycling in apple trees. Hortic. Rev. 4:204–246.

Toselli, M., J.A. Flore, B. Maragoni and A. Masia. 1999. Effects of root-zone temperature on nitrogen accumulation by non-bearing apple trees. J. Hort. Sci. Biotech. 74:118–124.

Toselli, M., J.A. Flore, C. Zavalloni and B. Maragoni. 2000. Nitrogen partitioning in apple trees as affected by application time. HortTechnology 10:136–141.

Tromp, J. and J.C. Ovaa. 1971. Spring mobilization of storage nitrogen in isolated shoot sections of apple. Physiol. Plant. 25:16–22.

Tromp, J. and J.C. Ovaa. 1973. Spring mobilization of protein nitrogen in apple bark. Physiol. Plant. 19:1–5.

Vasilieva, G., N. Mironomva and A. Glyanko. 1999. Low above-zero temperature effect in the root zone on nitrate reductase activity and nitrate content in pea organs with stage of growth. J. Plant Nutr. 22:967–976.

Weih, M. and P.S. Karlsson. 1999. The nitrogen economy of mountain birch seedlings: implications for winter survival. J. Ecol. 87:211–219.

Weinbaum, S.A., I. Klein, F.E. Broadbent, W.C. Micke and T.T. Muraoka. 1984. Effects of time of nitrogen application and soil texture on the avaibility of isotopically labeled fertilizer nitrogen to reproductive and vegetative growth of mature almond trees. J. Am. Soc. Hort. Sci. 109:339–343.

Westwood, M.N. 1988. Temperate-zone pomology. Timber Press, Portland, OR, pp 1–40, 109–128.

Wilson, Jr., C.E., P.K. Bollich and R.J. Norman. 1998. Nitrogen application timing effects on nitrogen efficiency of dry-seeded rice. Soil Sci. Soc. Am. J. 62:959–964.

Yemm, E.W. and E.C. Cocking. 1955. The determination of amino acids with ninhydrin. Analyst 80:209–213.